REMARKS

Reconsideration and allowance of the subject application is solicited.

Claim 1 is amended above to recite the limitation that the DSM strain 6601 is identical in its genome to DSM 6601 Escherichia coli strain containing plasmids pMut1 and pMut2. Support for this can be found in the disclosure for instance at page 2, lines 1-3, and page 3, lines 34-37. Claim 2 is amended in steps b), c) and d). Step b) now recites "introducing the *sacB* gene into the plasmids obtained in step a) so as to produce a pMut1 plasmid carrying a resistance gene and a *sacB* gene, and a pMut2 plasmid carrying a resistance gene and a *sacB* gene." This clarifies that what is produced are two altered pMut plasmids. This is supported by page 2, line 24 – page 3, line 3, and the Examples. Step c) now recites "introducing the altered pMut1 and pMut2 plasmids obtained in step b) into the [[E.]] Escherichia coli strain DSM 6601 substantially simultaneously, and cultivating the strain obtained thereby under conditions in which the naturally occurring ~~plasmids~~ pMut1 and pMut2 plasmids are displaced by the altered pMut1 and pMut2 plasmids obtained in step b)". This language streamlines the claim language and what is occurring in this step. The language "substantially simultaneously" clarifies that the introduction of both plasmids was done simultaneously. This is supported by the specification at page 2, lines 21-23, and the Examples. In Step d), it is recited: "cultivating the clones obtained in step c) that substantially only permit the growth of bacteria that lack the *sacB* gene, so that the Escherichia coli containing the altered pMut1 and pMut2 plasmids do not grow, and thereby is produced a plasmid-free clone of Escherichia coli strain DSM 6601." This clarifies that the final product is a plasmid-free clone of Escherichia coli strain DSM 6601. This is supported by page 3, lines 21-26 and page 4, line 1-4. No new matter is introduced by these revisions to claims 1 and 2, and entry is requested. By entry of this Amendment, claims 1-6 will be pending.

In the December 23, 2008 Office Action, claim 1 is rejected under 35 U.S.C. §103(a) as obvious over Blum-Oehler et al (WO 98/44134) in view of Trevors et al.

Claims 2-6 are rejected under 35 U.S.C.§103(a) as obvious over Uraji et al. in view of Blum-Oehler et al (WO 99/44134) in view of Trevors et al., and in view of Alexeyev et al.

The Examiner cites Blum-Oehler for the disclosure of Nissle 1917 (presumed to be the same as DSM 6601), but acknowledges that this reference does not teach a plasmid-free clone of DSM 6601. However, the Examiner argues that Blum-Oehler teaches that the Nissle 1917 strain has two plasmids (pMut1 and pMut2) that are cryptic and without benefit to the host. The Examiner cites Trevors et al. for teaching methods for removing bacterial plasmids. Combining Blum-Oehler and Trevors, the Examiner has found claim 1 obvious. Regarding the Alexeyev et al., the Examiner notes that this reference is cited to teach the use of a tetracycline cassette in a plasmid, and is combinable with the other three references to make claims 2-6 obvious.

We have amended independent claim 1 above to clearly distinguish from the cited art. While we disagree with the Examiner's maintenance of the art rejections, we make this amendment to claim 1 in order to advance prosecution.

Blum-Oehler teaches the DSM 6601 strain containing plasmids pMut1 and pMut2. Our claim covers a plasmid-free clone of Escherichia coli strain DSM 6601, which strain is identical in its genome to DSM 6601 Escherichia coli strain containing plasmids pMut1 and pMut2.

In the May 26 Office Action, the Examiner stated that the Trevors reference is cited mainly to teach the desirability of curing plasmids from bacteria. While this may be generally true, what Trevors cannot be cited to teach is our particular claimed E. coli strain: a plasmid-free DSM 6601 where the genome is identical to the wild-type DSM 6601. It appears to be agreed upon that Trevors et al. is a review article that describes several methods for "curing" bacteria from plasmids, and that, none of these methods equate to the actual method the inventors used to construct the claimed plasmid-free strains. As noted in our specification at page 2,

> It turned out in the exhaustive investigations that led to the present invention that plasmid-free clones of strain DSM 6601 cannot be prepared at all with normal genetic engineering methods or can be prepared only with great difficulty so that special paths must be taken in order to generate such clones. Since the wild type of the strain has two plasmids of different sizes in addition to its genomic DNA, the elimination of these plasmids must take place in several steps that take place in part in parallel.

In other words, it took significantly more than mere routine procedures. The

general teaching of Trevors is not enough to have lead someone having ordinary skill in this art – with Blum-Oehler and Trevors in hand – to reasonably expect to achieve without undue experimentation, a plasmid-free clone of Escherichia coli strain DSM 6601, which strain is identical in its genome to DSM 6601 Escherichia coli strain containing plasmids pMut1 and pMut2.

As stated in our description at page 4, first paragraph, one major advantage of our new strain is that, with the condition of the loss of the plasmids plus the fact that there was no change of the genomic DNA, the strain can be readily used as a cloning vehicle.

> Thus, they can be safely used in the laboratory as host cell for the cloning and expression of a plurality of genes and proteins. Experiments with strain DSM 6601 ΔpMut1/2 have shown that it is an especially good accepter for foreign DNA when the latter is integrated into its own plasmids present in isolated form, that is, therefore its own plasmids function as cloning vectors for the foreign DNA. Furthermore, since they are derived from a non-pathogenic strain, they can be used for the treatment of disturbances of the gastrointestinal tract in animals and humans. To this end they can be transformed, if desired with foreign genes that further the adhesion of the bacteria to the mucosa such as, e.g., adhesines that further the adhesion of the bacteria, optionally host-animal specifically, to the mucosa of, e.g., cattle and/or swine and thus hinder or prevent the growth of other pathogenic microorganisms. (Specification at page 4).

Our strain of claim 1 has features and advantages that are neither taught nor suggested by the combination of Blum-Oehler and Trevors. Reconsideration of this rejection is respectfully requested.

The method of claims 2-6 are similarly situated – they depend from claim 1 and have the limitation that the new Escherichia coli strain DSM 6601 strain is identical in its genome to DSM 6601 Escherichia coli strain containing plasmids pMut1 and pMut2. This alone is enough to distinguish from the cited art.

In addition, claim 2 is amended to clarify in step c) that the altered pMut1 and pMut2 plasmids obtained in step b) are introduced substantially simultaneously into the Escherichia coli strain DSM 6601. As described in the Examples, the introduction of both plasmids, pMUT1Tc-sacB and pMUT2Kn-sacB, **simultaneously** into E. coli was performed by electroporation followed by plating on agar plates containing Tetracycline

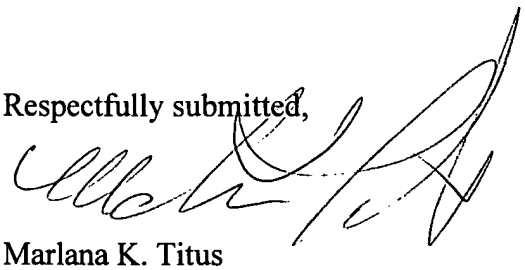
as well as Kanamycin. This approach allowed the elimination of both cryptic plasmids of E. coli in one step – which is something the cited art does not teach, and an advantage.

The claimed method of producing such a new strain is itself new and not disclosed by Uraji in combination with any of the other cited references. Unlike to the technique employed by Uraji et al. (conjugation) the plasmids carrying an antibiotic resistance gene and the sacB gene were introduced into E. coli simultaneously, by electroporation. This technique allowed the simultaneous translocation of two plasmids (e.g., pMUT1Tc-sacB and pMUT2Kn-sacB) into the E. coli and subsequent selection for transformants on agar plates containing both antibiotics (e.g., tetracycline and kanamycin). For electroporation, the E. coli was cultivated in LB broth to an optical density of 0.5 at wavelength of 600nm, washed 3 times with sterile, ice-cold 10 % (vol/vol) glycerol, and concentrated 10-fold. 40 µl of this concentrate were then electroporated with 1 µl of salt-free plasmid DNA in a cuvette at 2,5 kV, 200 Ω und 25 µF. This is in sharp contrast to the transfer of plasmids by conjugation into the bacterial strain to be cured as was reported by Uraji et al. In fact, the inventors even attempted their new method because earlier approaches to cure E. coli from both plasmids by heat, SDS or EthBr were all unsuccessful.

It is submitted that someone having ordinary skill in the art would not have found our method of claims 2-6 obvious with any or all of the four cited references in hand. The claimed method for curing *E. coli* strain DSM 6601 from both plasmids is not a technique that is merely a routine combination of the methods in the aforementioned publications which for an ordinary person skilled in the art would have found obvious. Reconsideration of this rejection is respectfully requested.

In summary, all of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 977-7227.

Respectfully submitted,



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